

INFLUENCES OF CALCIUM SOURCES, CONCENTRATION OF THE CEMENTATION SOLUTION AND TYPE OF SAND ON MICROBIAL INDUCED CARBONATE PRECIPITATION

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ABSTRACT

MICP technology is relatively one of the new technology emerge in the field of improving the soil. Therefore, this technique needs more intensive research before it becomes usable in widespread.

This research aims to study the effect of calcium source type, concentration of the cementation solution, the soil type, the use of cementation solution consists of a combination of calcium sources, on the calcium carbonate content, the crystal formation, the porosity, and the strength.

Set of laboratory tests were conducted, including porosity, calcium carbonate content, unconfined compressive strength and microscopy investigation (SEM). The results presented in this study revealed that using the cementation solution composed of calcium acetate and calcium chloride, which is made of egg shells and urea with MICP technique can improve the mechanical properties of the fine sand. Also, the use of a cementation solution containing variety mixtures of calcium sources did not show any noticeable difference in improving the properties of treated sand compared with sand treated with a cementation solution containing one type of calcium. The use of low concentrations of cementation solution provides a greater improvement in the engineering properties of the treated soil.

KEYWORDS: *Microbial-Induced Calcite Precipitation (MICP), Calcium Chloride, Calcium Acetate, UCS Test, Sporosarcina Pasteurii & Porosity*

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1. INTRODUCTION

For the time being construction on problematic soils is unavoidable because of the huge rhythm of city developments which led to the growing deficiency of land worldwide. Problematic soils are commonly characterized by low strength and high compressibility (Kazemian, Prasad, Huat, & Barghchi, 2011). Consequently, development on the problematic soils is apt to intense geohazards including settlement deformation, soil erosion, and landslides. Studies about soil improvement technicalities could be found profusely. The important attributes of soil improvement include: improving the shear strength of soil, reducing the time during which the settlement takes place, reducing potential for total and differential settlement, reducing potential for liquefaction in saturated fine sand or hydraulic fills, reducing the permeability of the soil, removing or excluding water from soil, etc (Kazemian & Huat, 2009). Traditionally, the criterion is to replace the vulnerable soil, low strength soil deposits, with engineered fills, also commonly referred to as "controlled" fills or "structural" fills. Nowadays, the use of chemical fillers is very frequently because of economic utility several kinds of chemical grouts are available. The most common are acrylate, sodium silicate, lignin, urethane, and Portland cement, asphalt, lime and resin

grouts. While many of these additives have proven successful the additives often modify the pH of the soils, and may contaminate the soils and groundwater (DeJong, Fritzges, & Nüsslein, 2006; Karol, 2003). Chemical grouts are also restricted under some conditions due to possibly toxic effects that have been detected with some of the unreacted grout components. Lately, with the growing interest in and awareness of environmental issues, it has been found that there is a significant shift towards remediation environmentally friendly and sustainable technologies. Harnessing biological processes to improve soil properties satisfies the societal demand for ground improvement in a natural manner. A very promising soil stabilization method using a biological process, which is known as microbial-induced calcite precipitation (MICP) has unfolded recently. MICP has been enabled through interdisciplinary research fields at the confluence of microbiology, geochemistry, and geotechnical engineering, to find natural treatments for soil improvement (DeJong, Mortensen, Martinez, & Nelson, 2010). MICP is a biological process that occurs in nature. It is an innovative alternative approach lies in the combined use of microorganisms, nutrients, and biological processes naturally present in subsurface soils to effectively improve their engineering properties (DeJong et al., 2006).

Le Métayer-Levrel (Le Metayer-Levrel, Castanier, Orial, Loubiere, & Perthuisot, 1999), Mitchell (Mitchell & Santamarina, 2005), Lian et al. (Lian, Hu, Chen, Ji, & Teng, 2006), DeJong et al. (2010) (DeJong et al., 2010), Achal, et al. (Achal, Mukherjee, & Reddy, 2011), Montoya (Montoya, 2012), Al Qabany et al. (Al Qabany & Soga, 2013), Shahrokhi-Shahraki, et al. (Shahrokhi-Shahraki, Zomorodian, Niazi, & O'Kelly, 2015), Jiang et al. (Jiang, Yoshioka, Yamamoto, & Soga, 2016) have conducted studies and researches on this comparatively new technique. These studies have led to the fashioning of solid ground for the subsequent developing on this pioneering technique as a practical solution for soft ground problems.

This research aims to study the effect of calcium source type, concentration of the cementation solution, the soil type, the use of cementation solution consists of a combination of calcium sources, on the calcium carbonate content, the crystal formation, the porosity, and the strength. In this research, section 2 discusses, the materials and methods of preparation of soil specimens, bacterial culture and cementation solution In addition to laboratory tests. Section 3 discusses, results and discussion. Section 4 discusses, conclusions.

2. MATERIALS AND METHODS

2.1 Preparation of Soil Specimens

Two types of poorly graded sand were selected for the current study. The first type was fine silica sand which remaining on the sieve with an opening size of 0.1 mm. While the second type was river sand taken from the Yangtze river bank. The poorly graded sand column was prepared by packing the dry sand (with a unit weight of 16 kN/m³ and 14 kN/m³, porosity of 37.67 % and 48.67 %, and pore void volume of about 33 ml and 43 ml, for silica sand and river sand respectively) into a polyvinyl chloride (PVC) column of 80 mm high and 37 mm inner diameter.

2.2 Bacterial Culture and Cementation Solution

The ureolytic bacterium used in the current study was *Sporosarcina pasteurii* (ATCC 11859). The ATCC 11859 was cultivated under sterile aerobic batch conditions in a yeast extract medium (20 g/l yeast extract, 10 g/l ammonium sulfate, 0.13 M Tris buffer, pH = 9). The optical density OD₆₀₀ of the bacterial culture varied between 1.4 and 1.5. The bio-cementation was conducted using four types of cementation solution, the first one is two concentrations of cementation solution consisting of equal moles of pure anhydrous calcium chloride (1 M, 111 g/l; 0.5 M, 55.5 g/l) and urea

(1 M, 60 g/l; 0.5 M, 30 g/l). The second type is two concentrations of cementation solution consisting of equal moles of pure calcium acetate monohydrate (1 M, 176 g/l; 0.5 M, 88g/l) and urea (1 M, 60 g/l; 0.5 M, 30 g/l). The third type prepared from urea (1 M, 60 g/l) and calcium chloride made of eggshells (1 M). The fourth type prepared from urea (1 M, 60 g/l) and calcium acetate made of eggshells (1 M).

2.3 MICP Procedure

Microbial induced carbonate precipitation for soil treatment was conducted using gravity-induced downward precipitation. Initially, the sand columns were divided into two groups. The first group was silica sand columns, and the second group was Yangtze river sand columns. The two groups were flushed with 33 ml for silica sand and 43 ml for Yangtze river sand bacterial culture, followed by 3 hours of retention time to allow the cells of bacteria installs itself in the soil granules. At this stage, each group was divided into six subgroups depending on the type of cementation solution. The first subgroup was flushed with a cementation solution made of CaCl_2 (eggshells) and urea, the second and third subgroups were flushed with a cementation solution made of pure CaCl_2 and urea (1 M, 0.5 M), the fourth subgroup was flushed with a cementation solution made of $\text{C}_4\text{H}_6\text{O}_4\text{Ca}$ (eggshells) and urea. While the fifth and sixth subgroups were flushed with a cementation solution made of pure $\text{C}_4\text{H}_6\text{O}_4\text{Ca}$ and urea (1 M, 0.5 M). In addition to the previous groups, another two subgroups of Yangtze river sand were prepared, the first was flushed with a cementation solution made of a mixture of calcium chloride and calcium acetate and urea (1 M), while the second was flushed with a cementation solution made of calcium chloride and urea (1 M) and calcium acetate and urea (1 M) in alternating order. The MICP reaction time was 24 h for cementation solution with concentration of (1 M) and 12 h with a concentration of (0.5 M). The flushing with cementation solution was repeated every 24 h for cementation solution with concentration of (1 M) and 12 h with a concentration of (0.5 M). The whole tests were performed at a temperature of $30^\circ\text{C} \pm 2^\circ\text{C}$. After 4 days of flushing with cementation solution, the treatment was stopped and the soil specimens were put in the oven at a temperature of 60°C for 7 days. The variables and details of the experimental combinations are tabulated in Table 1.

Table 1: Combinations of MICP Treatment

Type of Soil	Type of Cementation Solution	Combination Abbreviation
Yangtze river sand	CaCl_2 (eggshells) and urea (1M)	A
	$\text{C}_4\text{H}_6\text{O}_4\text{Ca}$ (eggshells) and urea (1M)	B
	CaCl_2 (AR) and urea (1M)	C
	$\text{C}_4\text{H}_6\text{O}_4\text{Ca}$ (AR) and urea (1M)	D
	CaCl_2 (AR) and urea (0.5M)	E
	$\text{C}_4\text{H}_6\text{O}_4\text{Ca}$ (AR) and urea (0.5M)	F
	$(\text{CaCl}_2 + \text{C}_4\text{H}_6\text{O}_4\text{Ca})$ and urea (1M)	G
	$(\text{CaCl}_2 \text{ or } \text{C}_4\text{H}_6\text{O}_4\text{Ca})$ and urea (1M) (alternate)	H
Silica sand	CaCl_2 (eggshells) and urea (1M)	I
	$\text{C}_4\text{H}_6\text{O}_4\text{Ca}$ (eggshells) and urea (1M)	J
	CaCl_2 (AR) and urea (1M)	K
	$\text{C}_4\text{H}_6\text{O}_4\text{Ca}$ (AR) and urea (1M)	L
	CaCl_2 (AR) and urea (0.5M)	M
	$\text{C}_4\text{H}_6\text{O}_4\text{Ca}$ (AR) and urea (0.5M)	N

2.4 CaCO_3 Content

To determine the amount of precipitated CaCO_3 in the soil specimens. The specimens were crushed, oven-dried and recorded the dry weights. The dry soil was soaked in HCl solution to dissolve precipitated CaCO_3 , then washed with

water and finally oven-dried and record the weights.

The difference between the weights before and after soaking in HCl considered to be the weight of the CaCO_3 precipitated in the specimen

2.5 Unconfined Compressive Strength

Unconfined Compressive Strength (UCS) tests were conducted according to the procedure reported in ASTM D2166 (ASTM, 2013) on bio-cemented soil specimens with selected diameter-to-height ratios of 1:2. The samples were flushed with deionized water (about four pore volumes) and dried at 60°C for 24 hours prior to UCS measurements. These tests were conducted to establish the relationship between the strength of the soil samples and its calcium source and soil type.

2.6 Microscopy Investigation

In order to study the calcium carbonate types, bonds and their distribution within the sand after treatments, the scanning electron microscope (SEM) was conducted.

3. RESULTS AND DISCUSSIONS

3.1 Porosity

The porosity can be defined as a measure of the volume of voids in soil to the total volume of the soil. These voids may be among grains of sand, silt, clay and organic matters in the soil or cavities of the soil. Usually, this open space filled with air or water or both. Hence, the precipitation of calcium carbonate in the pore spaces will result in a reduction of the pore space volume.

The porosity was calculated by measuring the difference between the mass of the sample. Where, the volume of water contained in a saturated column of known volume can indicate porosity. The mass of saturated sample minus the oven-dry mass of the sample, divided by the density of water, gives the volume of water. The volume of water divided by the original volume of the sample gives porosity (Equation 1).

$$n = \frac{M_{\text{sat}} - M_{\text{dry}}}{\rho_w V_t} \quad (1)$$

Where

n : porosity M_{sat} : mass of saturated sample M_{dry} : mass of the dry sample ρ_w : density of water V_t : volume of sample

The untreated sand column sample was prepared at same initial density for the purpose of comparing the porosity of the samples before and after treatment. The pore volume of the specimens was decreased as a result of the MICP treatment. The decrease in porosity of treated samples were from 48.67% (for all the river sand specimens before treatment) to 44.91%, 44.31%, 45.11%, 44.63%, 44.52%, 45.31%, 45.12%, 44.01% for groups A - H respectively, and from 37.67% (for all the Silica sand specimens before treatment) to 34.95%, 34.33%, 34.33%, 35.06, 34.23%, 34.57% for groups I - N respectively. Table 2 shows the porosity of soil specimens before and after cementation by MICP technique and the amount of the decrease at the end of treatment duration relative to the original porosity.

Table 2: Porosity before and after Treatment

Specimen Group	Initial Porosity %	Final Porosity %	Decrease in Porosity %
A	48.67	44.91	7.75
B	48.67	44.31	8.98
C	48.67	45.11	7.34
D	48.67	44.63	8.36
E	48.67	44.52	8.57
F	48.67	45.31	6.93
G	48.67	45.12	7.34
H	48.67	44.01	9.60
I	37.67	34.95	7.22
J	37.67	34.33	8.86
K	37.67	34.33	8.87
L	37.67	35.06	6.92
M	37.67	34.23	9.14
N	37.67	34.57	8.23

It was clear that there is diversity in the decrease of porosity between the samples. This is due to the different concentrations and types of soil and cementation chemical solutions used in the treatment. Also, it can be interpreted as a result of the amount of bacterial cells that have been absorbed into the soil particles. Where it is, in general, the samples which absorbed greater amount of bacteria cells were more capable to form greater amount of calcium carbonate compared with samples that absorb less amount of bacteria cells.

3.2 Calcium Carbonate Content

The calcium carbonate content (i.e., gram/gram calcite per sand) of the samples of the two types of soil which treated with different calcium sources are shown in Figure 1.

Figure 1 shows that the river sand samples (groups A- H) showed greater ability to precipitation of calcium carbonate from those shown by silica sand samples (groups I-N). This happened regardless of the type, source and concentration of calcium. Where the highest calcium carbonate content of the river sand was 0.173 in group B and less content was 0.155 in group A, while higher calcium carbonate content of silica sand was 0.131 in the group (N) and less content was 0.116 in group (K). In general, the average content of calcium carbonate for all groups of river sand was 0.165, and the average content of calcium carbonate for all groups of silica sand was 0.136.

After the end of treatment with two types of calcium chloride (1M), calcium carbonate content was (0.155), (0.163), (0.125) and (0.116) in groups A, C, I, K, respectively. These values showed that the bacteria cells were able to produce approximately the same amount of calcium carbonate in the river sand samples (groups A and C), and the same thing in silica sand samples (groups I, K) for both types of cementation solution. The same thing happened with the groups treated two types of calcium acetate, where the calcium carbonate content was (0.173), (0.162), (0.120) and (0.122) in groups B, D, J, L, respectively.

Also, Figure 1 shows that the groups E, F, M, N, which treated with a cementation solution of calcium chloride and urea or calcium acetate and urea concentration of 0.5 M, showed calcium carbonate content slightly higher than the calcium carbonate content of the corresponding groups from the same type of soil which treated with a cementation solution concentration of 1 M. Where the average calcium carbonate content of samples treated with cementation solution concentration of 0.5 were 0.170 and 0.120 for river sand and silica sand respectively, compared with the average calcium carbonate content of samples treated with cementation solution concentrate 1M which were 0.163 and 0.120 for river sand

and silica sand respectively.

From the above, it is clear that the type and source of calcium in the cementation solution did not have a great significance in the precipitated amount of calcium carbonate. The factors that impacted significantly on the precipitated amount of calcium carbonate were mainly, the soil type (especially the porous of soil), secondly, the concentration of the cementation solution.

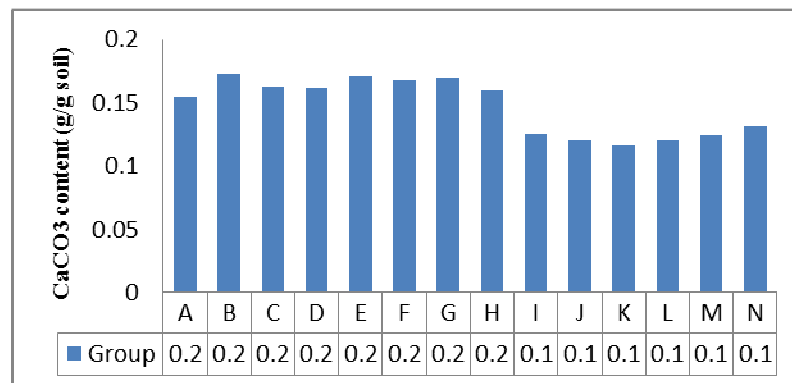


Figure 1: Effect of Calcium Source and Soil Type on the Calcium Carbonate Content

3.3 Unconfined Compressive Strength of Treated Soil

The effect of MICP on the strength of treated soil under different experimental procedures was determined by applying UCS test. The UCS values were determined as the highest deviatoric stresses.

The UCS of the samples of the two types of soil which treated with different calcium sources are shown in Figure 2.

Figure 2 shows that the treated silica sand samples became stronger than the treated river sand samples, regardless of the type and concentration of the cementation solution used for treatment, where the average unconfined compressive strength was 656.17 and 442.31 in the silica sand and river sand samples, respectively.

The samples in groups A, B, I and J that have been treated by using a cementation solution content calcium source (calcium chloride and calcium acetate) made of eggshells showed results very close to the results of the samples C, D, K and L which have been treated with a cementation solution containing pure calcium chloride and calcium acetate (AR). Except group J, this group showed strength less than the strength of group L. Anyway, this result was unexpected because calcium acetate made of egg shells that were used in treatment the sample J was left salt deposits on the upper surface of the sample shortly after the end of the injection process, and with repeated injections the amount of salt increases on the surface (Figure 3). This led to slow penetration of the cementation solution into the soil, and consequently, heterogeneity in the distribution of calcium carbonate sediments. This happened with group J and did not happen to group L because of the low permeability of silica sand (group J) compared with the permeability of river sand (group L).

Also, the results shown in Figure 2 indicate that the samples using calcium acetate (groups B, D, F, J, L and N) as the calcium source possess a slightly higher strength than those using calcium chloride (groups A, C, E, I, K and M). Except group J, this group showed strength less than the strength of group I, for the reasons which have been reported previously. These results agreed with the findings of the Zhang et al. (Zhang, Guo, & Cheng, 2014).

Also, Figure 2 showed that the samples in groups E, F, M and N that has been treated with a cementation solution of concentration (0.5 M) have higher strength from those in the other groups which were treated with a cementation solution of concentration (1 M). This indicates that the input chemical concentration potentially has an effect on the precipitation pattern. Kunst et al. (Kunst & Rapoport, 1995) reported that the bacteria growth under a salt stress condition has a negative effect on the production of hydrolysis enzyme. Calcium chloride and calcium acetate, which is the main components in the cementation solution, are salts that may contribute to the salinity of cementation solution. Therefore, It is not recommended for the MICP treatment using a very high concentration of cementation solution.

Finally, for the purpose of exploring the effect of using different types of calcium sources at the same time for soil treatment, it was prepared two groups (G and H). Group G was treated by using a mixture containing calcium acetate and calcium chloride and urea of concentration (1 M). While group H has been treated using alternating injections of two cementation solution, the first one contains calcium chloride and urea while the second containing calcium acetate and urea.

The results showed that the strength of the groups G and H did not differ much from the strength of the other groups that have been using one type of calcium sources in treatment them. In general, group G showed greater improvement in strength compared to the group H.

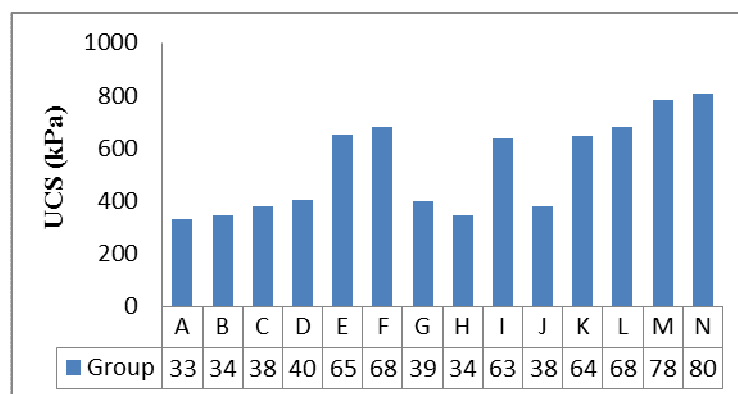


Figure 2: Influence of Calcium Source, Cementation Solution Concentration and Soil Type on the Strength

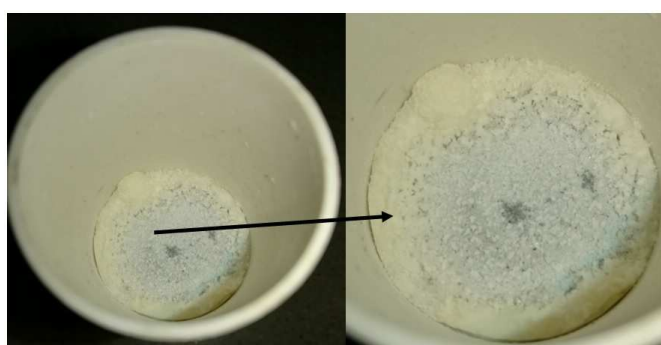


Figure 3: The Salt Deposits on the Upper Surface of the Samples of Group J

3.4 Scanning Electron Microscopy (SEM)

Figure 4 shows the image of the electron microscope of the samples of river sand and silica sand in groups A, C, I and K. For the river sand samples, rhombohedral crystals with smooth surfaces could be observed as a characteristic for calcite. While for the silica sand samples, the hexahedral crystals with rough surfaces as a characteristic for calcite could be observed in the case of the samples treated with cementation solution composed of calcium chloride (egg shells) and urea,

and the hexahedral crystals with smooth surfaces of the samples treated with cementation solution composed of calcium chloride (analytical grade) and urea. This means that the same type of cementation solution produced two different forms of calcite crystals (rhombohedral crystals, hexahedral crystals), depending on the type of treated soil.

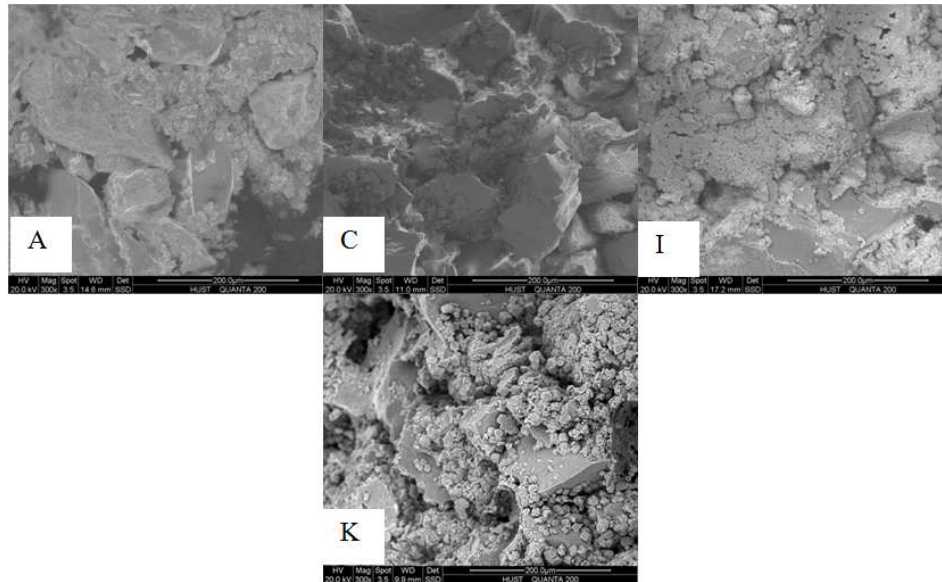


Figure 4: Samples Treated with 1M of CaCl_2 (Eggshells and AR) Cementation Solution and Different Soil Types

From SEM images Figure 5, the sand granules in groups M and E were fully covered by calcium carbonate. The strength of the groups M and E were higher than strength of other groups, although the calcium carbonate content does not change by a large margin. This can be explained by that the strength of the cemented column was due to the point-to-point contacts of calcium carbonate crystals, and because the calcium carbonate crystals formed by the concentration of 0.5 M solution was distributed regularly and more homogeneous than the crystals of concentration 1 M, therefore; the calcium carbonate crystals of concentration 1M covered the sand particles but the point-to-point contacts binding the particles together was weaker. Also, rhombohedral crystals were observed in group E, while in group M hexahedral crystals with smooth surfaces were observed.

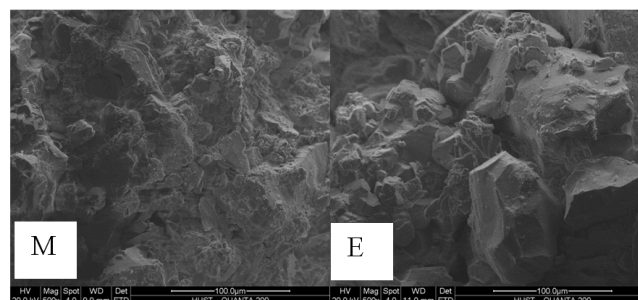


Figure 5: Samples Treated with 0.5 M of CaCl_2 (AR) Cementation Solution and Different Soil Types

Figure 6 shows the image of the electron microscope of the groups B and J. These groups were treated with cementation solution contain calcium acetate made of eggshells and urea. In previous research the crystal type of the MICP of the samples treated with calcium acetate is mainly aragonite. The acicular morphology is the typical characteristics of the aragonite (Zhang et al., 2014) (Figure 7a). De Muynck et al. (De Muynck, Cox, De Belie, & Verstraete, 2008) discovered that the crystal morphology of calcium carbonate can be influenced by the composition of cementation solution.

Figure 1 consists of three SEM images labeled (a), (b), and (c). Image (a) shows a dense layer of needle-like crystals. Image (b) shows a cluster of larger, more irregular crystals. Image (c) shows a single, large, well-defined needle-like crystal labeled 'Aragonite'. A white box in (a) and an arrow point to the crystal in (c). Scale bars are 20 μm.

4. CONCLUSIONS

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